Estrus synchronization and pregnancy rates in beef cattle given CIDR-B, prostaglandin and estradiol, or GnRH

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Abstract — Two experiments were conducted to determine estrous response and pregnancy rate in beef cattle given a controlled internal drug release (CIDR-B) device plus prostaglandin $F_{2\alpha}$ (PGF) at CIDR-B removal, and estradiol or gonadotropin releasing hormone (GnRH). In Experiment I, crossbred beef heifers received a CIDR-B device and 1 mg estradiol benzoate (EB), plus 100 mg progesterone (E + P group; $n = 41$), 100 µg gonadotropin releasing hormone (GnRH group; $n = 42$), or no further treatment (Control group; $n = 42$), on Day 0. On Day 7, CIDR-B devices were removed and heifers were treated with PGF. Heifers in the E + P group were given 1 mg EB, 24 h after PGF, and then inseminated 30 h later. Heifers in the GnRH group were given 100 µg GnRH, 54 h after PGF, and concurrently inseminated. Control heifers were inseminated 12 h after onset of estrus. The estrous rate was lower ($P < 0.01$) in the GnRH group (55%) than in either the E + P (100%) or Control (83%) groups. The mean interval from CIDR-B removal to estrus was shorter ($P < 0.01$) and less variable ($P < 0.01$) in the E + P group than in the GnRH or Control groups. Pregnancy rate in the E + P group (76%) was higher ($P < 0.01$) than in the GnRH (48%) or Control (38%) groups. In Experiment II, 84 cows were treated similarly to the E + P group in Experiment I. Cows received 100 mg progesterone and either 1 mg EB or 5 mg estradiol-17ß (E-17ß) on Day 0 and either 1 mg of EB or 1 mg of E-17ß on Day 8 (24 h after CIDR-B removal), in a 2 × 2 factorial design, and were inseminated 30 h later. There were no differences among groups for estrous rates or conception rates. The mean interval from CIDR-B removal to estrus was 44.2 h, $s = 11.2$. Conception rates were 67%, 62%, 52%, and 71% in Groups E-17ß/E-17ß, E-17ß/EB, EB/E-17ß, and EB/EB, respectively. In cattle given a CIDR-B device and estradiol plus progesterone, treatment with either EB or E-17ß effectively synchronized estrus and resulted in acceptable conception rates to fixed-time artificial insemination.

Résumé — Synchronisation des œstrus et taux de gestation chez des bovins de boucherie traités à l'aide d'un dispositif vaginal à libération contrôle de progestérone (CIDR-B), de prostaglandine et d'œstradiol ou de gonadolibérine. Deux expériences ont été menées afin de déterminer l'induction de l'œstrus et le taux de gestation chez des bovins de boucherie ayant reçu un dispositif interne à libération contrôlée de médicaments (CIDR-B) ainsi que de la prostaglandine $F_{2\alpha}$ (PGF) au retrait du CIDR-B et de l'œstradiol ou de la gonadolibérine (GnRH). Dans l'expérience I, des génisses de boucherie de race croisée ont reçu au jour 0 un dispositif CIDR-B et 1 mg de benzoate d'œstradiol (BO) ainsi que 100 mg de progestérone (groupe O + P; $n = 41$), 100 µg de gonadolibérine (groupe GnRH; $n = 42$) ou pas d'autres traitements (groupe témoin; $n = 42$). Au jour 7, les dispositifs vaginaux (CIDR-B) ont été retirés et les génisses ont reçu de la PGF. Les génisses des groupes O + P ont reçu 1 mg de BO, 24 h après la PGF et ont été inséminées 30 h plus tard. Les génisses du groupe GnRH ont reçu 100 µg de gonadolibérine et de la PGF 54 h plus tard, en même temps que l'insémination. Les génisses du groupe témoin ont été inséminées 12 h après le début de l'œstrus. Le taux d'œstrus était plus bas ($P < 0.01$) dans le groupe GnRH (55 %) que dans les groupes O + P (100 %) ou témoin (83 %). Le temps moyen entre le retrait du CIDR-B et l'œstrus était plus court ($P < 0.01$) et moins variable ($P < 0.01$) que dans les groupes GnRH et témoin. Les taux de gestation dans le groupe O + P (76 %) était plus élevé ($P < 0.01$) que dans les groupes (GnRH (48 %) ou témoin (38 %). Dans l'expérience II, 84 vaches ont été traitées de façon semblable à celles du groupe O + P de l'expérience I. Les vaches ont reçu 100 mg de progestérone et, soit 1 mg de BO, soit 5 mg d'œstradiol-17ß (O-17ß) au jour 0 et soit 1 mg de BO ou 1 mg de O-17ß au jour 8 (24 h après le retrait du CIDR-B),

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dans une analyse factorielle 2 × 2, et ont été inséminées 30 h plus tard. Il n'y avait pas de différence parmi les groupes aux niveaux des taux d'œstrus ou de conception. Le temps moyen entre le retrait du CIDR-B et l'œstrus était de 44,2 h, s = 11,2. Les taux de conception étaient respectivement de 67 %, 62 %, 52 % et 71 % dans les groupes O-17ß/O-17ß, O-17ß/BO, BO/O-17ß et BO-BO. Chez les bovins ayant reçu le dispositif CIDR-B ainsi qu'œstradiol et progestérone, un traitement avec soit du BO, soit du O-17ß, a effectivement synchronisé les œstrus et il en est résulté un taux de conception acceptable avec l'insémination artificielle à temps déterminé.

(Traduit par docteur André Blouin)

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Introduction

Recent approaches to estrous synchronization and fixed-time artificial insemination (AI) involve control of the luteal phase (endogenous and/or exogenous source of progestins), synchronization of follicular wave emergence, and synchronization of the preovulatory LH surge and ovulation (1). One protocol employing this approach is the Ovsynch regimen, consisting of 2 gonadotropin releasing hormone (GnRH) treatments (8 to 9 d apart), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment 30 to 48 h before the 2nd GnRH treatment, and timed-AI 0 to 24 h after the 2nd GnRH treatment (2). The 1st GnRH treatment usually increases peripheral progesterone concentrations by inducing ovulation of a dominant follicle (3) and, thereby, synchronizes emergence of a new follicular wave. The 2nd GnRH treatment synchronizes the preovulatory luteinizing hormone (LH) surge and ovulation. However, 8.7% and 11.8% of cows have been reported to be detected in estrus between the 1st GnRH and PGF injections for 6- and 7-d programs, respectively (4). Exogenous progestin treatment would be expected to minimize the expression of estrus between the 1st GnRH and PGF treatments. In that regard, short-term progestin treatments have been successfully used to synchronize estrus (5). Furthermore, estrogens have been shown to synchronize both follicle wave emergence (6–8) and the preovulatory LH surge (9), and may be used instead of GnRH for these purposes.

The purpose of the present study was to determine the estrous response and pregnancy rate in beef cattle given a controlled internal drug release (CIDR-B) device and PGF at CIDR-B removal, and then estradiol or GnRH to induce follicle wave emergence and to synchronize ovulation for fixed-time AI.

Materials and methods

Experiment I

Postpubertal, cross-bred (Simmental-Angus-Gelbvieh) beef heifers (n = 125) received a CIDR-B device (Vetrepharm Canada, London, Ontario) at random stages of the estrous cycle (device insertion = Day 0). Heifers were randomly allocated to receive: 1 mg of estradiol benzoate (EB) and 100 mg of progesterone (Sigma Chemical Company, St. Louis, Missouri, USA) dissolved in 2 mL of canola oil (E + P group; n = 41); 100 µg gonadorelin acetate (Cystorelin, Merial Canada, Victoriaville, Québec) (GnRH group; n = 42); or no further treatment (Control group; n = 42). On Day 7, CIDR-B devices were removed and heifers were given 500 µg cloprostenol (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, Québec). Heifers in the E + P group were given 1 mg EB, 24 h after PGF, and were inseminated 30 h later (54 h after PGF). Heifers in the GnRH group were given 100 µg GnRH, 54 h after PGF, and concurrently inseminated. Control heifers were inseminated 12 h after onset of estrus. All hormone solutions were injected, IM. Estrus detection devices (Kamar Heatmount Detector; Kamar, Steamboat Springs, Colorado, USA) were glued onto the sacral region of the heifers at the time of CIDR-B removal. Heifers were observed for signs of mounting behavior and the mount detectors were examined for evidence of activation (red color), twice daily for 5 d after CIDR-B removal. The onset of estrus was defined as the 1st observation period at which the detector device had been activated or the heifer was observed in standing estrus. Pregnancy diagnosis was conducted with a real-time, B-mode scanner (Aloka SSD-500; Instruments for Science and Medicine, Edmonton, Alberta) equipped with a 7.5-MHz linear array transducer, 30 d after CIDR-B removal.

Estrous rate was defined as the proportion of heifers detected in estrus, conception rate was defined as the percentage of inseminated heifers that became pregnant, and pregnancy rate was defined as the percentage of the total number of heifers in the group that became pregnant. For the interval from PGF treatment to estrus, analysis of variance and Bartlett's test were used to compare means and variances, respectively. If there was a significant effect of group on means, differences were located with a least significant difference multiple range test. Chi-squared analyses were used to detect differences among treatments for estrus, conception, and pregnancy rates. All statistical analyses were conducted with a current computer program for statistical analysis (Statistix Student Version, v. 2.0; Analytical Software, Tallahassee, Florida, USA).

Experiment II

Eighty-four, suckled crossbred beef cows that were at least 50 d postpartum were treated similarly to the E + P group in Experiment I. Cows received 100 mg progesterone and either 1 mg of EB or 5 mg of estradiol-17ß (E-17ß; Sigma Chemical Company) on Day 0 and either 1 mg of EB or 1 mg of E-17ß on Day 8 (24 h after CIDR-B removal and PGF treatment), in a 2 × 2 factorial design. On Day 7, the CIDR-B devices were removed and cows were given PGF. Estrous detection was done by twice-daily observations. Cows were artificially inseminated 30 h after the 2nd estradiol injection (54 h after PGF treatment and CIDR-B removal), whether they showed estrus or not. Pregnancy diagnosis was conducted by rectal palpation 45 d after PGF treatment.

Table 1. Estrous, conception, and pregnancy rates in Control heifers or those treated with estradiol benzoate and progesterone (E + P) or GnRH in a CIDR-B-based estrus synchronization program with PGF at CIDR-B removal

End points	Control	E + P	GnRH
n	42	41	42
PGF to estrus			
Mean, s (h)	65.1^{b}, 19.8^{y}	48.6^{a}, 2.6^{x}	63.7^{b}, 22.5^{y}
Range (h)	48 to 120	48 to 60	48 to 108
Estrous rate	35/42 (83%)[c]	41/41 (100%)[a]	23/42 (55%)[b]
Conception rate	16/35 (46%)[b]	31/41 (76%)[a]	20/42 (48%)[b]
Pregnancy rate	16/42 (38%)[b]	31/41 (76%)[a]	20/42 (48%)[b]

[ab]Means with different superscripts are different ($P < 0.01$)
[xy]Variances with different superscripts are different ($P < 0.01$)
s = standard deviation

Table 2. Response of cows treated with a CIDR-B device and given estradiol-17ß (E-17ß) or estradiol benzoate (EB) plus 100 mg progesterone at CIDR-B insertion and either E-17ß or EB 24 h after PGF treatment and CIDR-B removal

End point	E-17ß/E-17ß	E-17ß/EB	EB/E-17ß	EB/EB
No. of cows	21	21	21	21
Estrous rate				
All cows	16/21 (76%)	19/21 (90%)	17/21 (81%)	17/21 (81%)
Cows with retained CIDR-B	12/16 (75%)	16/18 (89%)	16/20 (80%)	15/19 (79%)
PGF to estrus				
Mean, s (h)	41, 6.1	46, 5.0	44, 5.9	46, 4.7
Range (h)	36 to 48	36 to 48	36 to 48	36 to 48
Conception rate[a]				
All cows	14/21 (67%)	13/21 (62%)	11/21(52%)	15/21 (71%)
Cows with retained CIDR-B	10/16 (63%)	11/18 (61%)	10/20 (50%)	14/19 (74%)

[a]Fixed-time insemination at 54 h after PGF treatment and CIDR-B removal
s = standard deviation
No significant differences were found among groups for estrous ($P = 0.67$) or pregnancy rates ($P = 0.61$)

Statistical analyses were conducted as described in Experiment I.

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

Results

Experiment I

Results are summarized in Table 1. The estrous rate was significantly different ($P < 0.01$) between the GnRH group (55%) and the E + P (100%) and Control (83%) groups. The mean interval from CIDR-B removal to estrus was shorter ($P < 0.01$) and less variable ($P < 0.01$) in the E + P group (48.6 h, $s = 2.6$) than in either the GnRH (63.7 h, $s = 22.5$) or Control (65.1 h, $s = 19.8$) groups. Conception rate in the E + P group (76%) was higher ($P < 0.01$) than in both the GnRH (48%) and Control (46%) groups (35 of 42 heifers in the Control group were observed in estrus and inseminated, and 16 became pregnant).

Experiment II

Although some cows lost their CIDR-B devices (11/84, 13%; no differences among groups), they were inseminated at a fixed time along with those that retained their CIDR-B devices. The results are shown in Table 2. There were no significant differences among groups for estrous rates or conception rates ($P = 0.67$ and $P = 0.61$, respectively). Estrous rate for all cows combined (69/84; 82%) was not different ($P = 0.83$) from only those with a retained CIDR-B (59/73; 81%). Similarly, there was no difference ($P = 0.82$) in conception rates between all cows combined and cows with a retained CIDR-B (53/84, 63% vs. 45/73, 62%).

Discussion

In Experiment I, all heifers in the E + P group were detected in estrus and 76% became pregnant to a fixed-time insemination. In the GnRH group, the conception rate to a fixed-time insemination (48%) was acceptable, but the estrous rate (55%) was approximately half of that in the E + P group. In another study in which GnRH was given 7 d before PGF treatment, behavioral estrus (after PGF treatment) was detected in 51.3% of cows (4). Treatment with GnRH induces LH release, which may cause premature luteinization of ovarian follicles (thereby decreasing peripheral estrogen concentrations) or induce ovulation before peripheral estrogen concentrations peak, accounting for the lower rate of estrous detection. The principal purpose of detecting estrus is to determine the appropriate time at which to inseminate in anticipation of ovulation. However, estrous detection, and, indeed, whether or not estrus is expressed, is unimportant, if ovulation is synchronized in all or most cattle. Conception rate in the Control group was nearly identical to that in the GnRH group; however, due to a moderate rate of estrous detection, some heifers were not inseminated and the overall pregnancy rate was somewhat less than expected. Obviously, when cattle are only inseminated following detection of estrus, the combination of a moderate estrus rate and a moderate conception rate results in a relatively low pregnancy rate.

In Experiment II, EB and E-17ß were equally effective in inducing a high rate of estrus and pregnancy. Historically, estrogens have been given at the beginning of a progestin treatment regimen to induce luteolysis (via induction of PGF release) (10). However, the high rates of estrus and pregnancy in cows that lost their CIDR-B devices in this experiment suggests that these doses (and/or forms) of estradiol did not induce luteolysis. More recently, it has been demonstrated that estrogen treatment causes regression of antral follicles and synchronizes emergence of a new follicular wave (6). Further, it has been shown that, in the absence of elevated progestin concentrations, estrogen treatment induces an LH surge, resulting in transient or incomplete suppression of the dominant follicle and delayed emergence of the next follicular wave (11). Therefore, on the possibility of encountering cows with regressed corpus luteum in a group of randomly cycling cows, we have consistently included progesterone with the 1st estrogen treatment. Estrogen treatment has been given after PGF treatment to increase the proportion of cattle in estrus and the synchrony of estrous behavior and ovulation (12). In other studies, when 0.5 mg or 1.0 mg of estradiol benzoate was given after CIDR-B removal, the proportion of heifers detected in estrus was higher than in control heifers that did not receive estradiol (13,14). In another study (9), treatment with EB (0.38 mg and 1.0 mg in heifers and cows, respectively), 24 to 30 h after progesterone removal, effectively induced estrus (estrous rate, 86% and 100%, respectively). Furthermore, estrogen treatment in the present study appeared to induce an estrus more characteristic of a spontaneous estrus (considerable clear mucus discharge and a cervical canal that was patent) compared with that in the GnRH treatment group.

Two doses of GnRH are given 7 d apart in the Ovsynch regimen and PGF is given just before the 2nd injection (15). The 1st injection has been reported to induce ovulation (18/20 cows and 13/24 heifers responded by ovulating) or luteinization, followed by the emergence of a new follicular wave in 2.1 d, $s = 0.31$ or 1.5 d, $s = 0.47$ in cows and heifers, respectively. The 2nd injection of GnRH is given to induce an LH surge and, thereby, synchronize ovulation. All 20 cows and 18 of 24 heifers ovulated between 24 and 32 h after the 2nd injection of GnRH, suggesting that the Ovsynch regimen may not be as efficacious in heifers (15). It seems that heifers that failed to ovulate following the 2nd GnRH treatment were in metestrus or early diestrus and did not undergo luteolysis in response to the PGF treatment. Therefore, if the 1st GnRH treatment does not induce ovulation and emergence of a new follicular wave, ovulation of a dominant follicle following PGF treatment and a 2nd injection of GnRH may be poorly synchronized. Indeed, Wiltbank (2) observed that up to 20% of heifers showed estrus before the PGF treatment in Ovsynch programs. In a recent study (3), GnRH treatment induced ovulation in 56% of heifers treated during the growing, static, or regressing phases of the dominant follicle of the 1st follicular wave, and emergence of a new follicular wave occurred only when treatment caused ovulation. We have also shown that insertion of a CIDR-B device prevents the expression of estrus between the 1st injection of GnRH and PGF treatment, and pregnancy rates to a fixed-time insemination were higher when a CIDR-B device was used in the Ovsynch program in heifers (16).

Estrogens in combination with progesterone administered at the beginning of a CIDR-B treatment resulted in synchronous emergence of a new follicular wave in both heifers and cows (17). The estrogens used in the present study (E-17ß and EB) have a shorter half-life in circulation than does either estradiol valerate (commonly included in norgestomet-based estrous synchronization protocols; 11) or estradiol cypionate (18). Treatment with the latter 2 formulations of estradiol induced emergence of a new follicular wave at inconsistent and prolonged intervals. It is noteworthy that E-17ß has a shorter circulating half-life than does EB. Following treatment with 5 mg E-17ß, peripheral estradiol concentrations returned to baseline within 48 h (11), whereas treatment with 5 or 10 mg of EB resulted in elevated concentrations of serum estradiol for approximately 72 and 96 h, respectively (19). Follicular wave emergence has been reported to occur 4.3 d after treatment of progestagen-treated cattle with 5 mg of E-17ß (7) and 5.4 d after treatment with 5 mg of EB (17).

Subsequent to the completion of these studies, Bridges et al (20) reported an experiment utilizing 139 lactating postpartum beef cows that were given a CIDR-B device and allocated into 3 treatment groups to receive 2 mg EB (Groups 1 and 2) or no EB (Group 3) at the time of device insertion. The CIDR-B devices were subsequently removed after 7 d (Group 1) or 5 d (Groups 2 and 3), and all cows were given 25 mg PGF at that time, plus 1 mg EB 30 h after device removal, and were inseminated 28 to 30 h later (58 to 60 h after device removal). Estrous rates were 93%, 87%, and 81% and pregnancy rates were 60%, 50%, and 51% for cows in Groups 1, 2, and 3, respectively. These results are comparable with those reported in the present study, suggesting that there may be considerable flexibility as to when the CIDR-B device may be removed without interfering with pregnancy rates to a fixed-time insemination.

In the present studies, both E-17ß and EB were highly efficacious in synchronizing estrus, and presumably ovulation, in a CIDR-B-based, fixed-time AI program in cattle. Further studies are needed to confirm the necessity of synchronizing follicular wave emergence and to precisely document the effects of length of CIDR-B treatment and intervals to a 2nd estradiol treatment on the time of ovulation, in order to optimize the time of insemination and pregnancy rates.

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